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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1656	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/596,933	ABRAHAM ET AL.	
	Examiner	Art Unit	
	SUZANNE M. NOAKES	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 July 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24* is/are pending in the application.

4a) Of the above claim(s) *19,20,23 and 24* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18,21 and 22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/02/2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of Group I, claims 1-18, 21 and 22 in the reply filed on 22 July 2010 is acknowledged. The traversal is on the ground(s) that the '212 patent does not teach or disclose all of the limitations of claim 1, namely that the cross-linked enzyme particle size is ranging from about 50 to 150 microns. In addition the claims/Groups are so close in scope that essentially they are the same classes and subclasses and thus easily searchable together. This is not found persuasive because the as noted below, Khalaf et al. teach that the crystals should be microcrystals, wherein this is understood in the art to generally by crystals less than 100 microns (see Margolin or Zelinski – both cited below). Thus, Khalaf et al. inherently does teach this limitation and thus unity of invention between the two groups is lacking.

The requirement is still deemed proper and is therefore made FINAL.

Status of the Application

2. Claims 1-24 are pending; Claims 19, 20, 23 and 24 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected subject matter, there being no allowable generic or linking claim. Thus, claims 1-18, 21 and 22 are subject to examination on the merits.

Information Disclosure Statement

3. The information disclosure statement (IDS) submitted on 02 October 2006 has been considered by the examiner. See initialed and signed PTO-1449.

Claim Objections

4. Claims 5, 10, 14, 17, and 18 are objected to because of the following informalities:

In claim 5, the indefinite article “a” should appear in the second line between ‘as’ and ‘saturated’.

In claim 10, the last comma is unnecessary.

In claim 14, the conjunction “and” should appear between ‘sorbitan trioleate’ and ‘ sorbitan tristerate’

In claim 17, the term “Protease” is unnecessarily capitalized.

In claim 18, the list should be separate by comma and not semi-colons.

Appropriate correction is required.

Claim Rejections - 35 USC § 112 – 2nd paragraph

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 2 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The phrase "namely glucoamylase" renders the claim

indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

7. Claims 11-15 and 22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is noted that the claims refer to, for example, wherein the surfactants are selected from various groups or have specific ratios. However, there are two different points in the method of claim 1 which utilize surfactants, e.g. in part (a) during the crystallization and in part (d) in the coating process. Thus, it is unclear which surfactant and at which point in the claim is being referenced.

Claim Rejections - 35 USC § 102/103

8. The following are quotations of the appropriate paragraphs of 35 U.S.C. 102 and 35 U.S.C. 103 that form the basis for the rejections under this section made in this Office action:

> 35 USC 102:
>
> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> 35 USC 103:
>
> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 1, 8, 9, 11, 15, 22 are rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Khalaf (US Patent 5,932,212 – cited on IDS) as evidenced by Zelinski et al. (Agnew. Chem. Int. Ed. Engl. 1997, Vol. 36, No. 7, pp. 722-724) or Margolin (Trends in Biotechnology, 1996, Vol. 14, pp. 223-230).

Khalaf et al. teach a method of making Cross-linked Enzyme Crystals (CLEL's) in the presence of organic solvents and surfactants wherein the activity of said CLEC is greater than the soluble enzyme.

The (CLEC) formulations which exhibit high activity and productivity as catalysts in chemical reactions which is greater than that of soluble or conventionally-immobilized enzymes. The crosslinked protein crystal formulations are produced by drying or lyophilizing crosslinked protein crystals in the presence of a surfactant and an organic solvent. (Col. 3, lines 23-40)

It taught that the crosslinked enzyme crystal formulations do not require large single crystals which are needed for X-ray diffraction analysis. Rather microcrystalline showers are suitable and rather preferred – see col., lines 9, 14-17, wherein it is known in the art the terms "microcrystals" or "microcrystalline" generally means less than 100 microns in size – as evidenced by, Zelinski et al., (p. 722, 1st col., 2nd paragraph) and Margolin (See p. 224, 1st col., 1st paragraph).

The organic solvents to be used are organic solvent or aqueous-organic solvent chosen which is compatible with the protein constituent of the crosslinked protein crystal, as well as the surfactant used to stabilize the crosslinked protein crystal.

Organic solvents may be selected from the group consisting of diols, polyols, polyethers, water soluble polymers and mixtures thereof. Examples of organic solvents include toluene, octane, tetrahydrofuran, acetone, and pyridine. Further examples include hydrophobic or polar organic solvents such as, water miscible or water imiscible solvents, diethylene glycol, 2-methyl-2,4-pentanediol, poly(ethylene glycol), triethylene glycol, 1,4-butanediol, 1,2-butanediol, 2,3,-dimethyl-2,3-butanediol, 1,2-butanediol, dimethyl tartrate, monoalkyl ethers of poly(ethylene glycol), dialkyl ethers of polyethylene glycol), and polyvinylpyrrolidone, or mixtures thereof (see col. 5, lines 38-55).

The surfactants to be used are chosen from a detailed screening process as explained in Examples 6-8 which ensures protein/enzyme and surfactant compatibility. It is further noted that by using surfactants in addition to the organic solvents, the activity of the CLEC's is much greater, e.g 19-100 times greater activity in organic solvents as compared to when there is no organic solvent present (see col. 5, lines 8-17). The following are a few examples of surfactants that can be used or at least screened for compatibility in the process but is not a comprehensive list of known surfactants:

Cationic surfactants include amines, amine salts, sulfonium, phosphonium and quarternary ammonium compounds and specifically, Methyl trioctylammonium chloride (ALIQUAT 336), N,N',N'-polyoxyethylene(10)-N-tallow-1,3-diaminopropane (EDT-20,'PEG-10 tallow);

Anionic surfactants include, for example, linear alkylbenzene sulphonate, alpha-olefin sulphonate, alkyl sulphate, alcohol ethoxy sulfate, carboxylic acids, sulfuric esters and alkane sulfonic acids with specific examples to include: TRITON QS-30 (Anionic octyl phenoxy polyethoxyethanol), Aerosol dioctyl sulfosuccinate (AOT), Alkyl Sodium Sulfate (Niaproof): Type-4 or Type-8, Alkyl (C9-C.sub.13) Sodium Sulfates (TEEPOL HB7); and

Non-ionic surfactants useful for stabilization include nonyl phenol ethoxylate, alcohol ethoxylate, sorbitan trioleate, non-ionic block copolymer surfactants, polyethylene oxide or polyethylene oxide derivatives of phenol alcohols or fatty acids with specific examples of non-ionic surfactants to include: Polyoxyethylene Ethers: 4 lauryl Ether (BRIJ 30), 23 lauryl Ether (BRIJ 35); Octyl Phenoxy polyethoxyethanol (TRITIONS): Tx-15, Tx-100, Tx-114, Tx-405, DF-16, N-57, DF-12, CF-10, CF-54; Polyoxyethylenesorbitan: Monolaurate (TWEEN 20); Sorbitan: Sesquioleate (ARLACEL 83), Trioleate (SPAN 85); Polyglycol Ether (Tergitol): Type NP-4, Type NP-9, Type NP-35, Type TMN-10, Type 15-S-3, Type TMN-6(2,6,8, Trimethyl-4-nonyloxypolyethylenoxyethanol andType 15-S-40.

See Col. 10, line 24 to col. 11, line 27.

It is noted that generally, in order to prepare crosslinked enzyme crystal formulations, the surfactant should be added to a crosslinked enzyme crystal-containing solution in an amount sufficient to allow the surfactant to equilibrate with and/or penetrate the crosslinked enzyme crystals. Such an amount is one which provides a weight ratio of crosslinked enzyme crystals to surfactant between about 1:1 and about

1:5, preferably between about 1:1 and about 1:2. The crosslinked enzyme crystals are contacted with surfactant for a period of time between about 5 minutes and about 24 hours, preferably between about 30 minutes and about 24 hours. Following that contact, the crosslinked enzyme crystal/surfactant combination may be dried in the presence of an organic solvent to form the crosslinked enzyme crystal formulation. - See col. 11, lines 28-57.

It is also taught that any cross-linking reagent can be used, although glutaraldehyde it preferred, however, a more comprehensive list can be found in the Pierce Catalog (see col. 9, line 55 to Col. 10, line 2).

One of the many specific examples taught is Example 1, wherein lipase is crystallized (e.g. a hydrolase) and cross-linked wherein said crystals are solvent tolerant, thermostable and shear resistant. In Example 1, *Pseudomonas cepacia* lipase is crystallized in the presence of 20 mM calcium acetate, 200 mM magnesium sulfate (e.g. suitable salts), 23% isopropanol (e.g. a co-solvent also known as 2-propanol which is “about 20%”) and 1% glucopan (a non-ionic surfactant) at pH 5.5 and a temperature of 12°C (e.g. “about 10°C”) for about 16 hours.

This was followed by cross-linking the formed crystals with glutaraldehyde in 100 mM Tris at pH 9.25 (e.g. “about 8”) for eight hours at room-temperature.

This was followed by washing of the crystals extensively.

The washed crystals were placed in storage buffer and exposed to the surfactant N,N',N-polyoxyethylene (10)-N-tallow-1,3-diaminopropane (EDT-20 PEG-10 tallow

aminopropylamine) together with 2-butanone to obtain surfactant:cross-linked enzyme crystals ratio of 1:1. This was followed by drying of said crystals.

It is also taught in Col 11., lines 58-62 that an alternative to drying the cross-linked enzyme crystals, the crosslinked enzyme crystal/surfactant combination may instead be lyophilized in the presence of an organic solvent and surfactant. Lyophilization may be carried out for a period of time between about 30 minutes and about 18 hours.

Thus, while Khalaf does not teach specifically in Example 1 the lyophilization of the final product, it would nonetheless therefore be obvious to one skilled in the art to simply substitute the process of lyophilization for drying as Khalaf specifically teach this.

Finally, also as noted above, the crystals as taught by Khalaf et al. are microcrystalline, e.g. less than 100 microns as evidenced by Zelinski and Margolin.

Thus, all of elements specifically recited in claims 1, 8, 9, 11, 15, 22 are explicitly taught by Khalaf et al.

10. Claims 1-18, 21 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Khalaf (US Patent 5,932,212 – cited on IDS) as evidenced by Zelinski et al. or Margolin as applied to claims 1, 8, 9, 11, 15, 22 above and further in view of Navia et al. (US 5,849,296 – cited on IDS), Solovicova et al. (Acta Cryst. (1997) - D53, 782-783) and Henrikson et al. (Protein Science, 2001, Vol. 10, pp. 108-115).

The teaching of Khalaf et al. are taught above. Khalaf et al., however, do not teach that the crystallized enzymes are glucoamylase or a plant peroxidase (instant

claims 2-4), the crystallizing salts are sodium or ammonium sulfates (instant claim 5), the buffer used for cross-linking of the glucoamylase or peroxidase (instant claims 6 and 7), that the cross-linking reagent is glutaraldehyde and starch dialdehyde (claim 10); the glucoamylase to organic solvent ratio (instant claim 16), the activity of said cross-linked enzyme crystal (instant claim 17), that the peroxidases are active in organic solvents (claim 18) or the precise conditions which horse radish peroxidase is active in (claim 21).

Navia et al. teach a method of cross-linking protein enzyme crystals (CLEC—which presently is a trademarked name) which is similar to that taught by Khalaf (with the exception that Kalaf adds surfactants which is taught to significantly increase the overall activity of the end product CLEC). It is taught that a protein such as an enzyme or antibody is immobilized by crosslinking crystals of the protein with a multifunctional crosslinking agent such as glutaraldehyde (see co. 3, lines 42-43) although any cross-linking agent can be used such as those found, for example, in the Pierce catalog (see col. 15, lines 45-48). The crosslinked protein crystals may be lyophilized for storage (See col. 16, lines 44-57). A preferred protein is an enzyme such as thermolysin, elastase, asparaginase, lysozyme, lipase (e.g. a hydrolase) or urease, although it is specified that *any* enzyme which has been crystallized previously can be readily utilized – see for example, Table 1 which is just "a sampling of enzymes that have already been crystallized" (it is noted that plant horse radish peroxidase and glucose oxidase (e.g. an oxidoreductase) are cited). It is further established that the skilled artisan will have to adjust the conditions for crystallization and also cross-linking and tailor each for

the specific enzyme of choice and also to produce microcrystals rather than macrocrystals. Thus, many different crystallization and/or cross-linking conditions, buffers, temperatures, times and pH's are established by the skilled artisan and this is done so with an expectation of success given the many different examples cited by Navia et al. which is performed on a variety of different enzymes in different crystallization, cross-linking conditions, pH's, temperatures and times. Some specific examples include, Example 3 which utilizes elastase crystallized and cross-linked in a 100 mM acetate buffer at pH 5.0 (e.g. about pH 4.5); Example 5 utilizes lipase crystallized and cross-linked in 50 mM Tris buffer at pH 7.0 (e.g. between 6.5 and 8.0).

It is further taught that the crosslinked enzyme crystals retain at least 91% activity after incubation for three hours in the presence of a concentration of Pronase™ that causes the soluble uncrosslinked form of the enzyme to lose at least 94% of its initial activity under the same conditions (per instant claim 17). A preferred enzyme: Pronase™ ratio is 1:40. Enzyme crystals that are crosslinked may be microcrystals. Crosslinked enzymes (or antibody) crystals may be used in an assay, diagnostic kit or biosensor for detecting an analyte etc. (see Abstract and claims 1-15).

Solovicova et al. teach the successful crystallization of recombinant active glucoamylase in 100 mM acetate buffer, pH 4.7-5.5 (e.g. about pH 4.5), Peg 8K (30-32%) at 20°C by vapor diffusion (see p. 782, 2nd col., Crystallization) – per instant claim 7.

Henrikson et al. teach the successful crystallization of a plant peroxidase by ammonium sulfate precipitating said peroxidase, dialysing against Tris, pH 7.5 and then

crystallizing by vapor diffusion at 4°C, using ammonium phosphate 0.2-0.6 M and 100 mM Tris, pH 7.5 (see Crystallization, bottom p. 113 to top of 114) – per instant claims 3-6.

Therefore it would have been obvious to one of ordinary skill in the art at the time the invention was made to produce the cross-linked enzyme crystals using the technique of Khalaf et al. which includes the advantageous use of surfactants (which increases the activity of the CLECL) and to utilize any known crystallized enzyme as suggested and outlined by Navia et al., for example in Table 1, horse radish peroxidase, such as glucoamylase or plant peroxidase as taught by Solovicova et al. and Henrikson et al., respectively. It would further have been obvious to optimize the precise conditions for the crystallization conditions including temperature, co-solvents (e.g. organic solvents), surfactants, pH, salts and time because both Navia and Khalaf et al. specifically state that the process must be optimized for each particular enzyme. Thus, using any known crystallized enzyme and optimizing in these combined processes is not novel because, 'Where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.' *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233,235 (CCPA 1955)." On skilled in the art would have considerable expectation in success of utilizing any enzyme such as a plant horseradish peroxidase or a glucoamylase because and finding the optimal conditions as instructed and guided by Navia et al. and Khalaf et al. given the extensive and detailed methodology described and the numerous successful working examples, especially given that the optimum conditions for the enzymes such

as glucoamylase and plant peroxidases had already been established by Solovicova et al. and Henrikson et al.

Conclusion

11. No claim is allowed.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUZANNE M. NOAKES whose telephone number is (571)272-2924. The examiner can normally be reached on 7.00 AM-3.30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SUZANNE M. NOAKES/
Primary Examiner, Art Unit 1656
01 September 2010